

For the screening of populations of polypeptides such as the altered variable region populations produced by the methods of the invention, immobilization of the populations of altered variable regions to filters or other solid substrate is particularly advantageous because large numbers of different species can be efficiently screened for antigen binding. Such filter lifts will allow for the identification of altered variable regions that exhibit substantially the same or greater binding affinity compared to the donor CDR variable region. Alternatively, if the populations of altered variable regions are expressed on the surface of a cell or bacteriophage, for example, panning on immobilized antigen can be used to efficiently screen for the relative binding affinity of species within the population and for those which exhibit substantially the same or greater binding affinity than the donor CDR variable region.

Another affinity method for screening populations of altered variable regions polypeptides is a capture lift assay that is useful for identifying a binding molecule having selective affinity for a ligand (Watkins et. al., (1997)). This method employs the selective immobilization of altered variable regions to a solid support and then screening of the selectively immobilized altered variable regions for selective binding interactions against the cognate antigen or binding partner. Selective immobilization functions to increase the sensitivity of the binding interaction being measured since initial immobilization of a population of altered variable regions onto a solid support reduces non-specific binding interactions with irrelevant molecules or contaminants which can be present in the reaction.

Another method for screening populations or for measuring the affinity of individual altered variable region polypeptides is through surface plasmon resonance (SPR). This method is based on the phenomenon which

5 occurs when surface plasmon waves are excited at a metal/liquid interface. Light is directed at, and reflected from, the side of the surface not in contact with sample, and SPR causes a reduction in the reflected light intensity at a specific combination of angle and
10 wavelength. Biomolecular binding events cause changes in the refractive index at the surface layer, which are detected as changes in the SPR signal. The binding event can be either binding association or disassociation between a receptor-ligand pair. The changes in
15 refractive index can be measured essentially instantaneously and therefore allows for determination of the individual components of an affinity constant. More specifically, the method enables accurate measurements of association rates (k_{on}) and disassociation rates (k_{off}).

20 Measurements of k_{on} and k_{off} values can be advantageous because they can identify altered variable regions or optimized variable regions that are therapeutically more efficacious. For example, an altered variable region, or heteromeric binding fragment
25 thereof, can be more efficacious because it has, for example, a higher k_{on} valued compared to variable regions and heteromeric binding fragments that exhibit similar binding affinity. Increased efficacy is conferred because molecules with higher k_{on} values can specifically
30 bind and inhibit their target at a faster rate. Similarly, a molecule of the invention can be more efficacious because it exhibits a lower k_{off} value compared to molecules having similar binding affinity. Increased efficacy observed with molecules having lower

5 koff rates can be observed because, once bound, the
molecules are slower to dissociate from their target.
Although described with reference to the altered variable
regions and optimized variable regions of the invention
including, heteromeric variable region binding fragments
thereof, the methods described above for measuring
associating and disassociation rates are applicable to
essentially any antibody or fragment thereof for
identifying more effective binders for therapeutic or
10 diagnostic purposes.

Methods for measuring the affinity, including
association and disassociation rates using surface
plasmon resonance are well known in the arts and can be
found described in, for example, Jonsson and Malmquist,
15 Advances in Biosensors, 2:291-336 (1992) and Wu et al.
Proc. Natl. Acad. Sci. USA, 95:6037-6042 (1998).
Moreover, one apparatus well known in the art for
measuring binding interactions is a BIAcore 2000
instrument which is commercially available through
20 Pharmacia Biosensor, (Uppsala, Sweden).

Using any of the above described screening
methods, as well as others well known in the art, an
altered variable region having binding affinity
substantially the same or greater than the donor CDR
25 variable region is identified by detecting the binding of
at least one altered variable region within the
population to its antigen or cognate ligand.
Additionally, the above methods can alternatively be
modified by, for example, the addition of substrate and
30 reactants, to identify using the methods of the
invention, altered variable regions having catalytic
activity substantially the same or greater than the donor
CDR variable region within the populations. Comparision,